

Truck-in-the-Park II: Chemical and Bioassay Analyses of Diesel and Biodiesel Particulate Matter

FINAL REPORT

By

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SUMMARY

The exhaust from diesel fuel combustion is known to contain a highly complex mixture of compounds, many of which are toxic. The combustion of biodiesel fuel, such as rapeseed ethyl ester (REE), also produces a complex mixture of compounds. Biodiesel fuel is made from plant oils or animal fats and can be used alone or blended with petroleum diesel to operate diesel engines. However, before it can be used as a fuel, the plant oil or animal fat usually undergoes a process called transesterification, a chemical reaction that produces glycerol and an ester of the oil. The glycerol must be removed before the ester can be used as a fuel in unmodified diesel engines. Biodiesel burns cleaner than standard diesel fuel and is a biodegradable, renewable energy source. The particulate and toxic compound emissions from the combustion of biodiesel fuel currently are under investigation.

One approach to this investigation is to use a short-term bioassay in conjunction with chemical analyses to evaluate the potential human health effects from the complex mixture of compounds in diesel and biodiesel emissions. Some bioassays measure damage to DNA. Such damage is referred to as genotoxic activity and is one component in the complex process of cancer development. One group of toxicologically important compounds present in diesel and biodiesel emissions is polycyclic aromatic hydrocarbons, or PAHs. Some PAHs are potent mutagens (cause DNA damage) in animals, for example, and are thought to cause cancer in laboratory animals and in humans.

In collaboration with the University of Idaho, the Montana Department of Environmental Quality, and the U.S. Department of Energy, we previously studied the toxic emissions in particulate matter collected from a 1995 Dodge 3/4 ton 4x4 pickup truck equipped with a Cummins 5.9 liter turbocharged diesel engine (Kado et al., 1996). The project was called "Truck-in-the-Park" since the vehicle was used exclusively in Yellowstone National Park and fueled only with rapeseed ethyl ester (REE) biodiesel produced from rapeseed oil (Peterson et al., 1995). PAHs were measured in particulate matter collected from the exhaust for the diesel engine chassis dynamometer. We also investigated the genotoxicity (DNA damaging capability) of the particulate extracts. Four types of fuel were tested in the vehicle: 1) 100 percent REE, 2) 100 percent D2 diesel fuel (control), 3) 20 percent REE + 80 percent D2 diesel, and 4) 50 percent REE + 50 percent D2 diesel. Emissions were collected under conditions controlled by a chassis dynamometer-dilution tunnel at the Los Angeles County Metropolitan Transit Authority (LACMTA) facility. The truck engine was initially tested with a low odometer reading of 3,700 mi. To investigate possible changes in the particulate and toxic compound emissions, the truck was re-tested in 1998 at the LACMTA facility after accumulating 86,600 miles. The emissions were also re-tested for PAHs and mutagenic activity using the same four fuel types, with and without the use of a catalytic converter.

As in the initial 1995 test, no engine modifications were made for any of the REE fuel combinations. Emissions testing was conducted by the University of Idaho, University of California, Davis, and the LACMTA staff at the LACMTA chassis dynamometer test facility. The EPA Dynamometer Driving Schedule for Heavy Duty

Vehicles was used for all emission testing (40 CFR, Part 86, Appendix 1, Cycle D) and consisted of two approximately equally timed periods for sample collection, designated as P1 and P2.

The biodiesel and diesel particulate extracts were analyzed for 18 different PAHs by GC/MS using selective ion monitoring (SIM). Deuterated isotopes (internal standards) were added to each sample extract to compensate for losses during sample preparation.

Bioassay experiments were conducted to determine the specific mutagenic potency of the particulate matter extracts. The bioassay that was used is the microsuspension assay, which is a simple modification of the Salmonella/ microsome test (Kado et al., 1983, 1986). Different amounts, or doses, of the particulate matter extracts were tested and the mutagenic potency was determined from the slope of the linear portion (best-fit line) of the dose-response curve. The total emissions of mutagenic activity also were calculated on the basis of the potency of the PM extracts, measured as revertants per mg, the total PM emissions (revertants per test), and the total number of miles traveled during the test (revertants per mile).

For each fuel tested, the particulate matter (PM) emission rate was calculated, with and without the use of a catalytic converter. For each fuel tested, the PM emissions with the catalyst were lower than those measured without the catalyst. For all fuels tested with catalyst, the PM emissions were similar and ranged from approximately 0.1 to 0.12 g/mile. In general, the PM emissions measured for all fuels without catalyst were approximately 100 percent higher than those measured when the catalyst was in use. The catalyst reduced PM emissions from D2 by 44 percent, from 50 percent REE

by 47 percent, and from 100 percent REE by 54 percent. There was a 28 percent reduction for the 20 percent REE fuel,. Without the catalyst, the D2 and 20 percent REE fuels had lower PM emissions than the 50 percent REE and 100 percent REE.

The PM filter samples collected for each fuel tested were quantitatively analyzed for semi-volatile and non-volatile PAHs. For the catalyst-equipped vehicle, semi-volatile PAH emissions generally were higher for 20 percent REE than for D2. Emissions of non-volatile PAHs, such as benz(a)anthracene to benzo(ghi)perylene, typically were higher for biodiesel and biodiesel blends than for D2. For example, benzo(a)pyrene emissions were 0.16 μg / mile from D2, 0.35 μg / mile from 20 percent REE, 0.36 μg / mile from 50 percent REE, and 0.39 μg / mile from 100 percent REE. Some PAHs, such as fluoranthene, pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, perylene, and benzo(ghi)perylene, had higher emissions in the REE biodiesel fuel than for D2, when tested with or without the use of a catalyst.

Comment [D1]:

Semi-volatile PAHs (naphthalene through pyrene) were measured from the vehicle with and without catalyst. Either with or without catalyst, emission rates of naphthalene, methyl naphthalenes, and biphenyl were higher for D2 than for any of the biodiesel fuels. These compounds are also known to escape collection and may not reflect the total amount of these compounds actually emitted. Slightly heavier molecular weight semi-volatile PAHs such as dimethylnaphthalene, for example, had similar or slightly higher emission rates for the REE fuels than for D2. The highest molecular weight semi-volatile PAHs, such as fluoranthene and pyrene, are typically emitted 50 percent in the vapor-phase and 50 percent in the particle phase. For the vapor-phase samples,

the emission rates for fluoranthene and pyrene were similar for the D2, 80 percent REE, and 100 percent REE fuels. The 50 percent REE fuel had the highest emission rates for these two compounds.

The cold start D2 emissions of the semi-volatile PAHs were higher than those measured for hot start D2, hot start 100 percent REE, and cold start 100 percent REE. For example, the emission rate of 2,6-dimethyl naphthalene was approximately 500 $\mu\text{g}/\text{mile}$ for the cold start D2, 200 $\mu\text{g}/\text{mile}$ for the hot start D2, and 2 $\mu\text{g}/\text{mile}$ for 100 percent REE. For the hot start cycles, the semi-volatile PAH emissions were generally higher for D2 fuel. For example, phenanthrene emissions from D2 were approximately 60 $\mu\text{g}/\text{mile}$, while the emissions from 100 percent REE were approximately 18 $\mu\text{g}/\text{mile}$.

The mutagenic activity of the particulate matter was investigated for each fuel type with and without metabolic enzymes added. The metabolic enzymes (labeled S9) are necessary for certain compounds such as the PAHs to be detected. The bioassay results for the 100 percent REE fuel indicate that the mutagenic activity of P2 cycle emissions (revertants per μg of PM), with and without S9 metabolic enzymes, were the lowest of all fuels and blends tested. The next most potent PM emissions were obtained from the 50 percent REE, followed by the D2 fuel. The 20 percent REE had the highest mutagenic potency of all fuel emissions tested. From the slopes of these dose-response curves, the specific mutagenic activity per mass of PM (μg of PM), was calculated. The emissions of mutagenic activity (revertants per mile) were calculated from the specific mutagenic activity (revertants per μg) and the emissions of PM (μg PM per mile).

For the PM collected from the vehicle equipped with a catalyst, the total mutagenic emissions were highest for 20 percent REE (approximately 11×10^6 revertants/mile for both +S9 and -S9) than for the other fuels. This is approximately double the mutagenic emissions for D2 [6×10^6 revertants/mile (+S9); 7×10^6 revertants/mile (-S9)] and 50 percent REE [6×10^6 revertants/mile (+S9); 8×10^6 revertants/mile (-S9)]. The lowest mutagenic emissions were observed for the 100 percent REE fuel (1×10^6 revertants per mile, +S9 and 2×10^6 revertants per mile, -S9) tested with and without catalyst.

The total mutagenic activity emissions for the vehicle without catalyst were also determined. The highest mutagen emission rates occurred when using the D2 fuel (approximately 5×10^6 revertants/mile for both \pm S9). The 20 percent REE, 50 percent REE and 100 percent REE all had similar mutagenic emission rates of approximately 2×10^6 revertants/mile (+S9 or -S9), which was also the approximate emission rate for the 100 percent REE with catalyst.

There are a number of similarities between results from emissions testing of the truck at 3,700 miles and 86,600 miles. For example, in both studies using the catalyst-equipped engine, the more chemically reactive PAHs such as benzo(a)pyrene were emitted at greater levels for the 100 percent REE fuel and some of the blended REE fuels than for the D2 fuel. In both studies, use of D2 fuel (hot start) without the catalytic converter resulted in the highest levels of PAHs measured per mile, except for benzo(a)pyrene and perylene, which had higher total emissions per mile using the REE and REE blended fuels than the D2 fuel.

In both studies with and without the use of a catalyst, the 100 percent REE emissions had the lowest genotoxic activity compared to the REE blends and the D2

fuel. The blended REE fuels tested without the use of a catalyst had lower mutagenic emissions than the D2 fuel.

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Glossary of Terms and Abbreviations

DCM	Dichloromethane
DMSO	Dimethylsulfoxide
GC/MS	Gas Chromatography/Mass Spectrometry
LACMTA	Los Angeles County Municipal Transit Authority
PAH	Polycyclic aromatic hydrocarbon
REE	Rapeseed ethyl ester

I. INTRODUCTION

Biodiesel fuel is made from plant oils or animal fats. Biodiesel fuel can be used alone to operate diesel engines or it can be blended with petroleum diesel. However, before it can be used as a fuel, the plant oil or animal fat must first undergo a process called transesterification. This chemical reaction produces a glycerol and an ester of the oil. The glycerol must be removed before the ester can be used as a fuel in unmodified diesel engines. The fuel is cleaner burning than standard diesel fuel, biodegradable, and renewable. The particulate and toxic compound emissions from the combustion of biodiesel fuel are currently under investigation. One group of toxicologically important compounds present in diesel and biodiesel emissions are polycyclic aromatic hydrocarbons, or PAHs. Some PAHs are potent mutagens (cause DNA damage) in animals, for example, and are thought to cause cancer in laboratory animals and in humans.

In collaboration with the University of Idaho, the Montana Department of Environmental Quality, and the U.S. Department of Energy, we studied the toxic emissions in particulate matter collected from a 1995 Dodge Cummins diesel 4x4 pickup truck (Kado et al., 1997). The project was called "Truck-in-the-Park" since the vehicle was used exclusively in Yellowstone National Park and was run entirely on rapeseed ethyl ester (REE) biodiesel produced from rapeseed oil (Peterson et al., 1995). PAHs were measured from particulate matter collected from the exhaust. We also investigated the genotoxicity (DNA damaging capability) of the particulate extracts. Four types of fuel were tested in the vehicle: 1) 100 percent REE, 2) 100 percent 2-D diesel fuel (control), 3) 20 percent REE + 80 percent 2-D diesel, and 4) 50 percent REE + 50 percent 2-D diesel. Emissions were collected under conditions controlled by a chassis dynamometer-dilution tunnel at the Los Angeles County Metropolitan Transit

Authority (LACMTA) facility. The truck engine was initially tested with a low odometer reading of 3,700 mi. To investigate possible changes in the particulate and toxic compound emissions, the truck was re-tested in 1998 at the LACMTA facility after accumulating 86,600 miles. The emissions were also re-tested for PAHs and mutagenic activity using the four fuel types that were tested in the first phase of the study.

II. MATERIALS AND METHODS

A. Vehicle, Test Cycle, and Fuel Testing Matrix

The test vehicle was a 1995 Dodge 3/4 ton 4x4 pickup truck equipped with a Cummins 5.9 liter turbocharged diesel engine. The odometer reading during the original test conducted in 1995 was 3,700 miles. For subsequent testing, the odometer reading was 86,660 miles. This Dodge truck is rated at 8,600 lbs Gross Vehicular Weight. As in the initial 1995 test, no engine modifications were made for any of the REE fuel combinations. Emissions testing was conducted by the University of Idaho, University of California, Davis, and the LACMTA staff at the LACMTA chassis dynamometer test facility. The EPA Dynamometer Driving Schedule for Heavy Duty Vehicles was used for all emission testing (40 CFR, Part 86, Appendix 1, Cycle D). This cycle duration is 1,060 seconds and consists of two approximately equally timed parts for sample collection, designated as P1 and P2. The fuel mixtures and the number of test cycles that were performed on the chassis dynamometer for the toxic emissions study, with and without a catalytic converter, are summarized in Table 1.

Table 1. Testing parameters for sample emissions collection.

Fuel	Start Type	No. Cycles	Catalytic Converter
100% REE*	Cold	1	±
100% REE*	Hot	2	±
100% Diesel	Cold	1	±
100% Diesel	Hot	2	±
20% REE*	Hot	2	±
50% REE*	Hot	2	±

* Rapeseed ethyl ester

B. Filter Sample Collection

The filters used throughout this study were 70 mm Teflon-coated glass fiber filters (T60A20, Pall-Gelman, Ann Arbor, MI.). The filters were pre-cleaned with methanol and dichloromethane. Each filter set consisted of a primary filter and a secondary filter placed in series. The filters were conditioned in a humidity- and temperature- controlled room for 24 hours before and after sampling. Filter tare and final weights were recorded.

The stainless steel filter holder unit consisted of upper and lower stages for placement of primary and secondary (backup) filters. A secondary filter was used to trap any particles that passed through the primary filter without being trapped. For each test cycle, two filter sets were obtained representing the P1 and P2 portions of the test cycle. The P1 and P2 filter samples were analyzed separately for PAHs and genotoxic activity.

C. PAH Analyses

The biodiesel particulate extracts were analyzed for 18 different PAHs. Deuterated isotopes (internal standards) were added to each sample extract. By analyzing for these internal standards, it is possible to compensate for losses during sample preparation. The biodiesel filters were sonicated in dichloromethane (DCM), filtered, concentrated, and the resulting sample extracts were analyzed by GC/MS using selective ion monitoring (SIM).

Each filter sample was extracted in pre-cleaned flasks to which 25 mls of DCM were added, followed by deuterated internal standards. After sonication for 20 min, the extract was transferred to a holding flask and the extraction was repeated three additional times with 20 ml of DCM. The entire extract in the holding flask was filtered through a Teflon filter (0.45 μ m Acrodisc, Gelman Sciences, Ann Arbor, MI.) and then concentrated to a final volume of 0.1 ml under a gentle stream of nitrogen.

1. Chemicals

Dichloromethane (OmniSolve, EM Science) was used throughout to preclean glassware and to extract filter samples. Naphthalene-d₈, acenaphthene-d₈, phenanthrene-d₁₀, chrysene-d₁₀, perylene-d₁₂ were from AccuStandard (New Haven, CT). All other deuterated standards were from Cambridge Isotopes Laboratories (Andover, MA). Benzo[e]pyrene and perylene were from Chem Services (West Chester, PA). The target PAH compounds and the corresponding deuterated internal standards are listed in Table 2 along with the target and qualifier ions used to identify and quantitate the analytes.

TABLE 2. Target PAHs and Internal Standards.

COMPOUND	TARGET AND QUALIFIER IONS
Naphthalene-d ₈ *	136, 68
Naphthalene	128, 129, 127
Acenaphthene-d ₁₀ *	162, 164, 160
Acenaphthylene	152, 153, 151
Acenaphthene	153, 154, 152
Fluorene-d ₁₀ *	176, 174, 177
Fluorene	166, 165, 167
Phenanthrene-d ₁₀ *	188, 94, 90
Phenanthrene	178, 179, 177
Anthracene-d ₁₀ *	188, 187, 97
Anthracene	178, 177, 179
Fluoranthene-d ₁₀ *	212, 106
Fluoranthene	202, 203
Pyrene-d ₁₀ *	212, 106
Pyrene	202, 200
Chrysene-d ₁₂ *	240, 120, 236
Benz[a]Anthracene	228, 229, 227
Chrysene	228, 229, 227
Benzo[b]Fluoranthene-d ₁₂ *	264, 132
Benzo[b]Fluoranthene	252, 253, 126
Benzo[k]Fluoranthene-d ₁₂ *	264, 132
Benzo[k]Fluoranthene	252, 253, 126
Benzo[a]pyrene-d ₁₂ *	264, 132
Benzo[e]Pyrene	252, 126
Benzo[a]Pyrene	252, 253, 126
Perylene-d ₁₂ *	264, 265, 260
Perylene	252, 126
Dibenz[ah]Anthracene-d ₁₄ *	292, 293
Indeno[1,2,3-cd]Pyrene	276, 275, 138
Dibenz[ah]Anthracene	278, 279, 139
Benzo[g,h,i]Perylene-d ₁₂ *	288, 144
Benzo[g,h,i]Perylene	276, 275, 138

* deuterated internal standard

2. Instrument Conditions

A Hewlett Packard 5890 Series II gas chromatograph (GC) interfaced to a HP 5970A mass selective detector was used for the chemical analyses. The GC was equipped with a 30 m x .25 mm ID J&W DB-5ms (.25 micron film thickness) fused silica capillary column. Helium (99.999% purity) was used as the carrier gas. The GC was run in a splitless mode with electronic pressure pulse programming. A temperature program was used in constant pressure mode with vacuum compensation. The MSD was run in selective ion monitoring (SIM) or electron impact modes.

D. Bioassay Analyses

Bioassay experiments were conducted to determine the specific mutagenic activity (potency) of the particulate matter extracts. The bioassay used throughout is the microsuspension assay as previously reported (Kado et al., 1983, 1986). Prior to bioassay testing, all extracts were resuspended in dimethylsulfoxide (DMSO) for addition to the assay. For all samples, the specific mutagenic activity is reported as the number of revertants per milligram (mg) of particulate matter. The specific mutagenic activity is determined from the slope of the linear portion (best-fit line) of the dose-response curve. The total emissions of mutagenic activity were also calculated on the basis of the potency of the PM extracts (revertants per mg), the total PM emissions (revertants per test), and the total number of miles traveled during the test (revertants per mile).

III. RESULTS

A. Particle Emissions

The PM emission rate data from all tests are illustrated in **Figure 1** for the catalyst-equipped vehicle and in **Figure 2** for the vehicle without the catalyst. Without the catalyst, the D2 and 20 percent REE fuels had lower PM emissions than the 50 percent REE and 100 percent REE. When the catalyst was used, PM emissions from D2 were reduced by 44 percent, with reductions of 47 percent for 50 percent REE and 54 percent for 100 percent REE. For the 20 percent REE fuel, there was a 28 percent reduction, which may be due to lower emissions without catalyst compared to the other fuels.

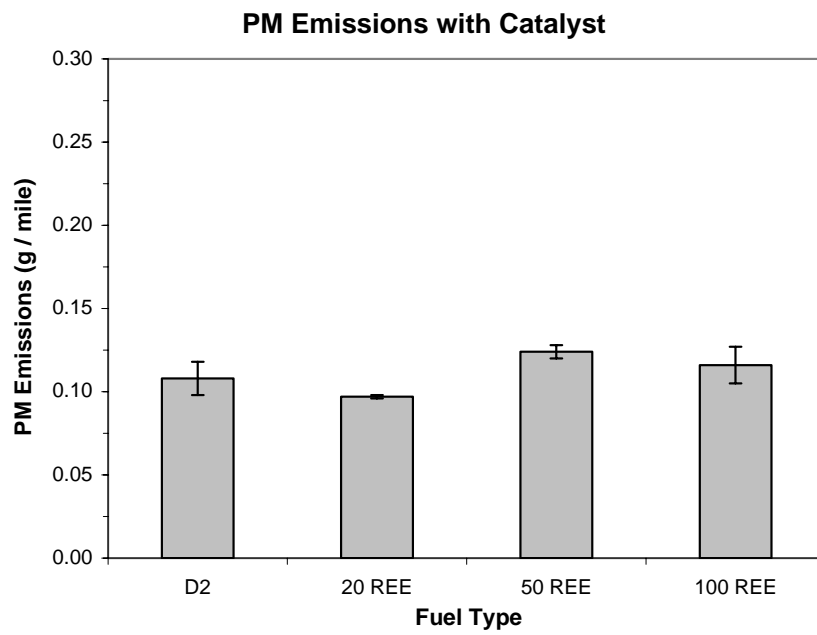


Figure 1. Particle emission rate data (+/- standard deviation) for the test fuels with catalyst-equipped vehicle.

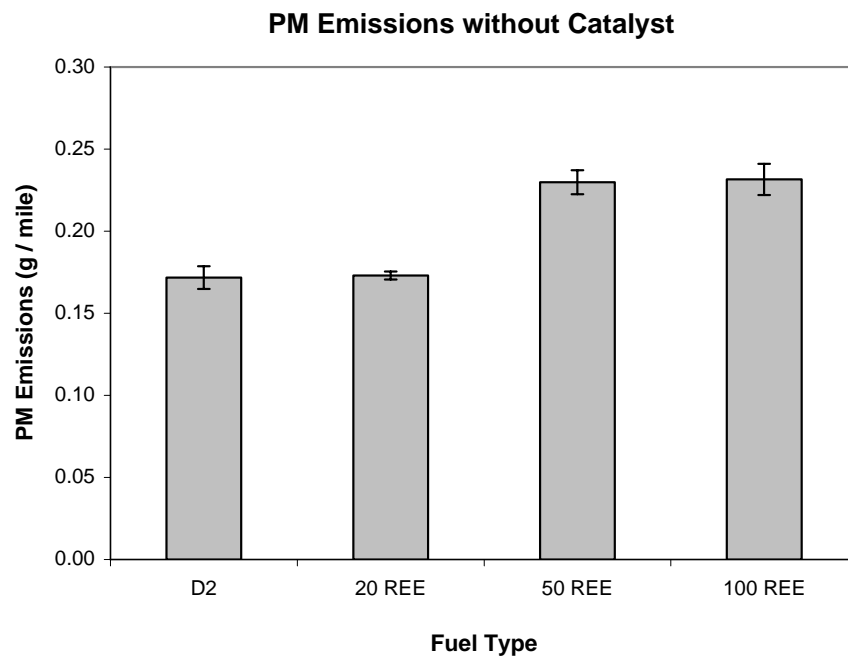


Figure 2. Particle emission rate data (+/- standard deviation) for the test fuels for vehicle without catalyst.

B. PAH Emissions

1. Particle-Associated PAH Emissions

Quantitative analyses for PAHs of particle samples collected on filters are as summarized in **Table 3** for the vehicle equipped with a catalyst, and in **Table 4** for the vehicle without the catalyst. For the catalyst-equipped vehicle, there were generally higher semi-volatile PAH emissions for 20 percent REE than for D2. There were typically higher emissions of “non-volatile” PAHs, such as benz(a)anthracene to benzo(ghi)perylene (**Tables 3 and 4**), for biodiesel and biodiesel blends than for D2. . For example, benzo(a)pyrene emissions were 0.16 µg / mile from D2, 0.35 µg / mile from 20 percent REE, 0.36 µg / mile from 50 percent REE, and 0.39 µg / mile from 100 percent REE.

Comment [D2]:

Table 3. PAH Emissions from catalyst-equipped vehicle.

PAH	<u>Emissions (µg / mile)</u>			
	D2	20% REE	50% REE	100% REE
fluorene	0.24	0.52	0.22	0.23
1-methyl phenanthrene	6.61	18.65	15.50	1.11
phenanthrene	10.60	29.15	28.81	5.22
anthracene	0.82	1.99	2.06	0.44
fluoranthene	5.06	9.98	10.89	16.90
pyrene	4.83	10.64	8.71	10.17
benz[a]anthracene	0.60	1.11	0.86	0.97
chrysene	0.86	1.89	1.64	1.40
benzo[b]fluoranthene	0.55	1.15	1.01	1.28
benzo[k]fluoranthene	0.20	0.23	0.19	0.36
benzo[e]pyrene	0.44	0.91	0.91	1.42
benzo[a]pyrene	0.16	0.35	0.36	0.39
perylene	0.07	0.11	0.09	0.16
indeno[1,2,3-cd]pyrene	0.18	0.31	0.33	0.53
dibenz[ah]anthracene	0.07	0.07	0.09	0.15
benzo[ghi]perylene	0.36	0.84	0.70	1.17

Table 4. PAH Emissions from vehicle not equipped with catalyst.

PAH	D2	<u>Emissions (µg / mile)</u>		
		20% REE	50% REE	100% REE
fluorene	0.11	0.04	0.07	0.03
1-methyl phenanthrene	13.77	9.95	16.44	12.20
phenanthrene	12.05	11.47	21.71	21.90
anthracene	1.96	1.53	3.69	1.78
fluoranthene	3.90	4.22	5.31	9.97
pyrene	8.55	8.99	10.37	10.10
benz[a]anthracene	1.41	1.04	1.11	0.86
chrysene	1.29	1.24	1.42	1.19
benzo[b]fluoranthene	0.99	1.04	1.13	0.93
benzo[k]fluoranthene	0.21	0.39	0.35	0.35
benzo[e]pyrene	0.72	0.98	0.94	0.80
benzo[a]pyrene	0.34	0.49	0.50	0.48
perylene	0.13	0.17	0.17	0.15
indeno[1,2,3-cd]pyrene	0.30	0.42	0.30	0.24
dibenz[ah]anthracene	0.05	0.15	0.12	0.09
benzo[ghi]perylene	0.67	1.01	0.75	0.56

2. Semi-Volatile PAHs

Semi-volatile PAHs (naphthalene through pyrene) were measured from the PUF samples acquired from the vehicle with and without catalyst. In general, and for the catalyst equipped vehicle as summarized in **Table 5**, there were higher emission rates of naphthalene, methyl naphthalenes, and biphenyl when using D2 than biodiesel fuels. These compounds are also known to escape collection on PUF, and therefore may not reflect the total amount these compounds actually emitted. Slightly heavier molecular weight semi-volatile PAHs such as dimethylnaphthalene, for example, are more efficiently trapped on the PUF. These compounds had similar or slightly higher emission rates for the REE fuels than for D2. The highest molecular weight semi-volatile PAHs, such as fluoranthene and pyrene, typically are 50 percent vapor-phase and 50 percent particle phase. For the PUF samples, the emission rates for fluoranthene and pyrene were similar for the D2, 80 percent REE, and 100 percent REE fuels. The 50 percent REE fuel, had the highest emission rates for these two compounds.

Semi-volatile PAH emissions data collected from the vehicle without catalyst from cold and hot start cycles using D2 and 100 percent REE are summarized in **Table 6**. The cold start D2 emissions were higher for most of the semi-volatile PAHs measured than for hot start D2, hot start 100 percent REE, and cold start 100 percent REE. For example, the emission rate of 2,6-dimethyl naphthalene for the cold start D2 was approximately 500 µg/mile, but only 200 µg/mile for hot start D2 and 2 µg/mile for hot start 100 percent REE. For the hot start cycles, use of the D2 fuel generally produced higher emissions of the semi-volatile PAHs. For example, phenanthrene emissions

from D2 were approximately 60 µg/mile, while the emissions from 100 percent REE were approximately 18 µg/mile (**Table 6**).

Table 5. Semi-volatile PAH emissions from catalyst-equipped vehicle.

Sample ID	CL100	HL104	HL106	HL110	HL113	HL101
Fuel type	100% REE	D2	D2	20% REE	50% REE	100% REE
Catalyst	Yes	Yes	Yes	Yes	Yes	Yes
Cycle type	Cold	Hot	Hot	Hot	Hot	Hot
Semi-Volatile PAH	Emissions (µg/mile)					
biphenyl	20.96	156.2	201.3	80.78	159.7	81.51
2,6-dimethyl naphthalene	2.14	137.7	178.6	109.2	182.7	110.5
acenaphthylene	21.50	20.57	26.72	28.44	38.92	28.56
acenaphthene	3.07	50.35	33.31	78.28	82.59	79.22
2,3,5-trimethyl-naphthalene	1.33	34.86	50.87	73.87	75.49	74.76
fluorine	5.63	21.49	29.01	35.66	48.01	36.02
1-methyl phenanthrene	0.86	2.42	4.39	5.17	11.31	5.22
phenanthrene	13.24	32.11	58.43	86.76	172.3	87.58
anthracene	3.33	4.29	8.26	6.13	110.1	6.15
fluoranthene	1.71	0.66	0.71	0.61	1.72	0.61
pyrene	1.86	0.60	0.61	0.49	1.17	0.49

Table 6. Semi-volatile PAH emissions without catalyst.

Sample ID	CL127	CL118	HL125	HL129	HL119	HL121
Fuel type	D2	100% REE	D2	D2	100% REE	100% REE
Catalyst	No	No	No	No	No	No
Cycle type	Cold	Cold	Hot	Hot	Hot	Hot
Semi-Volatile PAH		Emissions (µg/mile)				
naphthalene	274.5	62.52	106.8	143.1	33.47	25.06
2-methyl naphthalene	546.0	10.87	301.3	345.6	7.94	6.39
1-methyl naphthalene	373.0	8.92	201.6	228.1	6.15	4.79
biphenyl	184.5	8.88	125.4	137.4	6.85	5.51
2,6-dimethyl naphthalene	509.3	2.34	189.1	207.9	2.47	1.90
acenaphthylene	49.31	14.55	29.65	29.12	9.39	7.61
acenaphthene	170.7	2.04	62.70	72.15	1.44	1.14
2,3,5-trimethyl-naphthalene	234.5	1.97	88.03	105.46	1.41	1.15
fluorine	65.88	3.18	33.02	39.70	1.93	1.51
1-methyl phenanthrene	11.66	3.13	7.21	9.37	1.26	1.61
phenanthrene	116.5	22.20	55.11	68.42	20.22	16.38
anthracene	25.78	2.36	8.12	8.67	3.80	3.67
fluoranthene	0.46	0.36	0.69	0.41	0.25	0.26
pyrene	0.92	0.34	0.81	0.62	0.20	0.23

C. Bioassay Analyses

The mutagenic activity of the particulate matter was investigated for each fuel type with and without metabolic enzymes added. Different doses of PM extract were tested and the resulting data were used to generate dose-response relationships as illustrated in **Figure 3**. The metabolic enzymes (labeled S9) are necessary for certain compounds such as the PAHs to be detected. The mutagenic activity per μg of PM for the 100 percent REE is lowest for activity tested with and without S9. The next most potent PM emissions were obtained from the 50 percent REE, followed by the D2 fuel. The 20 percent REE had the highest mutagenic potency of all fuel emissions tested. From these dose-response curves, the specific mutagenic activity per mass of PM (μg of PM), is then calculated from the slopes of the dose-response curves. The emissions of mutagenic activity (revertants per mile) are calculated from the specific mutagenic activity (revertants per μg) and the emissions of PM (μg PM per mile).

The total emissions of mutagenic activity are illustrated in **Figures 4 and 5** for the fuels tested with and without catalyst. For the PM collected from the vehicle equipped with a catalyst, the total mutagenic emissions were highest for 20 percent REE (approximately 11×10^6 revertants/mile for both both + and - S9) than the other fuels as seen in **Figure 4**. This is approximately double the mutagenic emissions for D2 [6×10^6 revertants/mile (+S9); 7×10^6 revertants/mile (-S9)] and 50 percent REE [6×10^6 revertants/mile (+S9); 8×10^6 revertants/mile (-S9)]. The lowest mutagenic emissions were from 100 percent REE, with 1×10^6 revertants/mile for +S9 and 2×10^6 revertants/mile for -S9.

The total mutagenic activity emissions for the vehicle without catalyst are illustrated in **Figure 5**. The highest mutagen emission rates occurred when using the D2 fuel (approximately 5×10^6 revertants/mile for both \pm S9). The 20 percent REE, 50 percent REE and 100 percent REE all had similar mutagenic emission rates of approximately 2×10^6 revertants/mile (\pm S9), which was also the approximate emission rate for the 100 percent REE with catalyst.

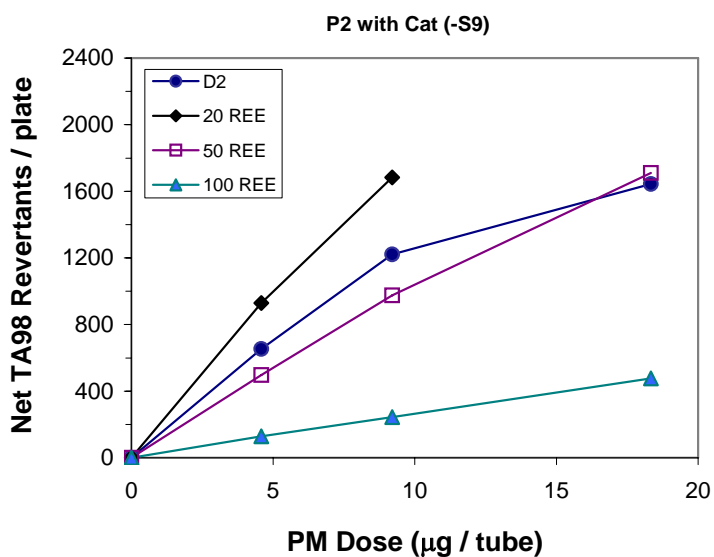
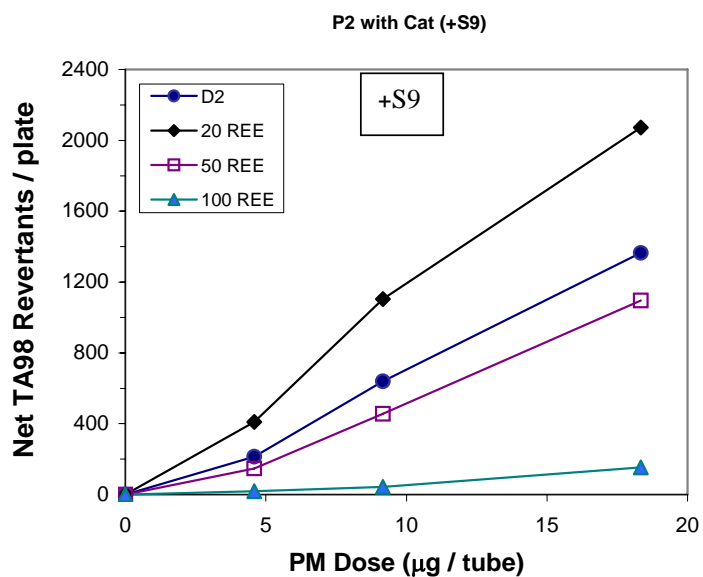


Figure 3. Dose-response curves for PM from each fuel type. The PM represented here was collected during the P2 portion of the testing cycle as described in the Methods section. The emissions are from the vehicle equipped with catalyst, and tested with and without metabolic enzymes (S9) added.

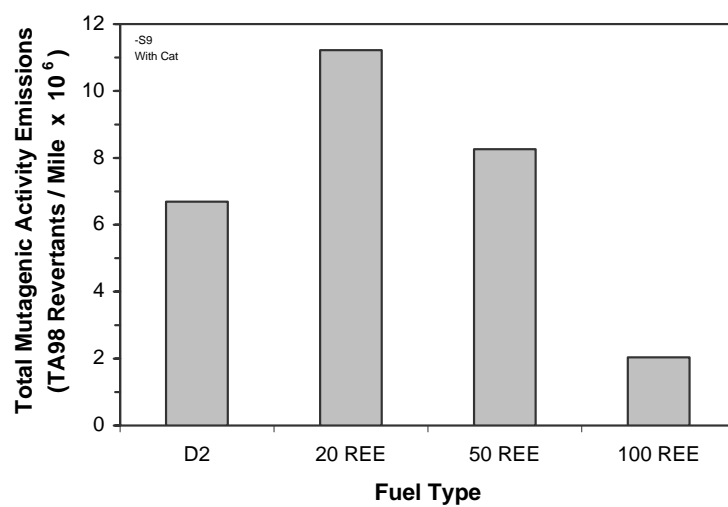
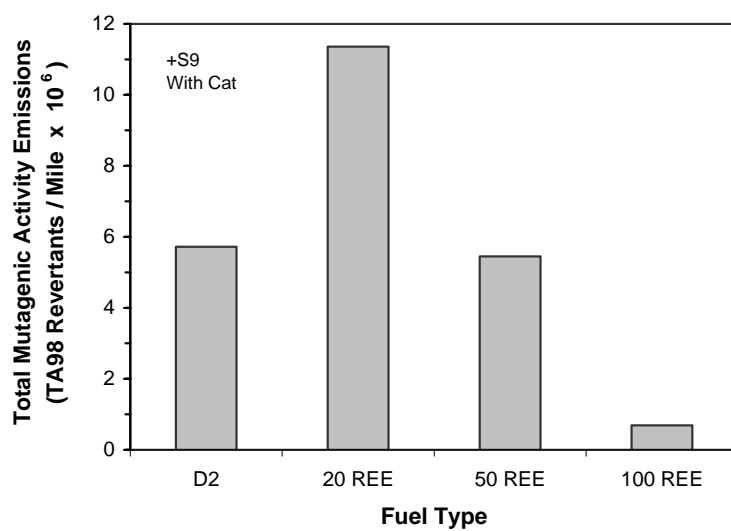


Figure 4. Total mutagenic emissions with catalyst.

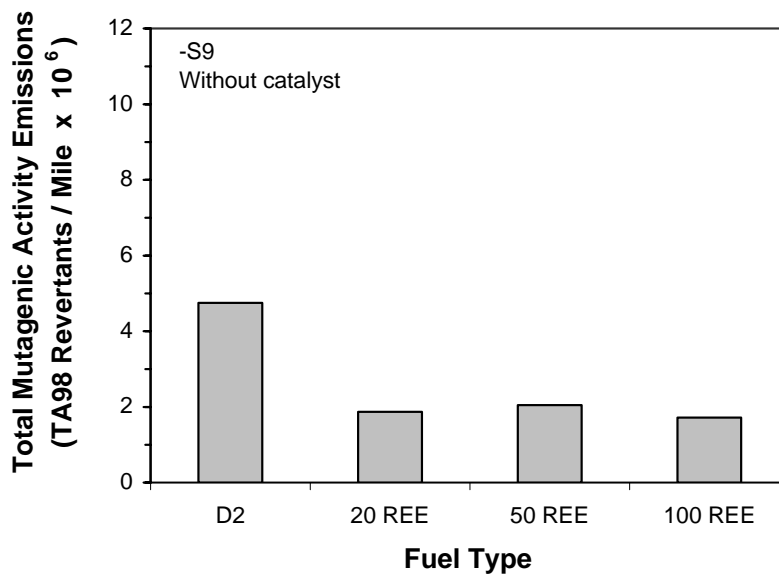
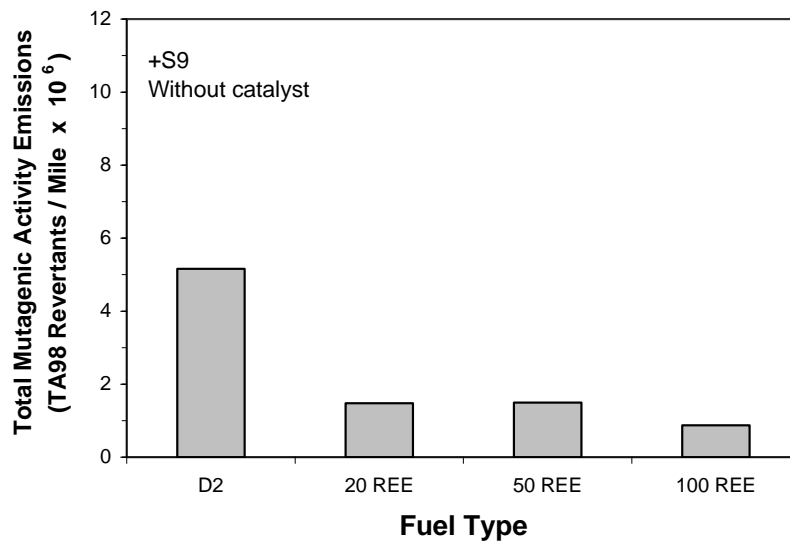


Figure 5. Total mutagenic emissions without catalyst.

IV. CONCLUSIONS

The PM, PAH, and mutagenic emissions from REE and its 20 percent and 50 percent blends with D2 were studied. For all fuels tested with catalyst, the PM emissions were similar and ranged from approximately 0.1 to 0.12 g/mile. The 50 percent REE and 100 percent REE fuels tested without a catalyst had greater PM emissions than the D2 and 20 percent REE fuels. The results for PM emissions from use of REE fuel with a catalyst were similar to those from use of D2. Without a catalyst, the PM emissions from use of the 50 percent and 100 percent REE fuels are higher than emissions from using D2 fuel. In general, the PM emissions measured for all fuels without catalyst were approximately 100 percent higher than those measured with catalyst.

For the 20 percent REE and 100 percent REE fuels tested with catalyst, semi-volatile PAH emissions were generally higher than for the D2 fuel. Semi-volatile PAH emissions for D2 fuel without catalyst were substantially higher than for REE and the REE blends. Emissions of non-volatile PAHs, such as benz(a)anthracene to benzo(ghi)perylene (see Table 3), were higher for REE and REE blends than for D2 when tested with catalyst. When tested without catalyst, emission rates of these same compounds were generally slightly higher than for D2, but not substantially different from one another.

The bioassay results for the 100 percent REE fuel indicate that the mutagenic activity of P2 cycle emissions (revertants per ug of PM) with and without S9 metabolic enzymes were the lowest of all fuels and blends tested. The highest emission of mutagenic activity was for the 20 percent REE fuel tested with and without catalyst.

The total emissions of mutagenic activity (revertants per mile) also were determined for each fuel, with and without the use of a catalyst. Total mutagenic emissions were highest for the 20% REE fuel with use of a catalyst (approximately 11×10^6 revertants per mile, with and without S9 metabolic enzymes). Mutagenic emissions for the 20% REE fuel were approximately double those measured for the D2 fuel (6×10^6 revertants per mile, +S9 and 7×10^6 revertants per mile, -S9) and the 50 percent REE fuel (6×10^6 revertants per mile, +S9 and 8×10^6 revertants per mile, -S9). The lowest mutagenic emissions were observed for the 100 percent REE fuel (1×10^6 revertants per mile, +S9 and 2×10^6 revertants per mile, -S9) tested with and without catalyst.

V. REFERENCES

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Appendix. Particle Filter Data

Test Run No.	C100		H101		H102		H103		H104	
Cycle type	Cold		Hot		Hot		Hot		Hot	
Fuel Type	100% REE		100% REE		100% REE		100% REE		D2	
Catalyst	Yes		Yes		Yes		Yes		Yes	
Cycle part	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
Primary Filter PM (mg)	1.171	0.994	0.264	0.623	0.289	0.628	0.315	0.613	0.270	0.608
Secondary Filter PM (mg)	0.118	0.152	0.063	0.13	0.084	0.132	0.052	0.12	0.066	0.116
Total Filter PM (mg)	1.289	1.146	0.327	0.753	0.373	0.76	0.367	0.733	0.336	0.724
Total grams of PM (g)	0.6709	0.5941	0.1704	0.3883	0.1941	0.3917	0.1911	0.3801	0.1749	0.3677
PM/ Mile	0.3906	0.1533	0.1002	0.1004	0.1144	0.1012	0.1134	0.0981	0.1036	0.0950
Distance (mile)	1.7176	3.8754	1.7006	3.8675	1.6967	3.8706	1.6852	3.8746	1.6882	3.8705
Test Total PM Emission	0.2262		0.1003		0.1052		0.1027		0.0976	

Test Run No.	H105		H106		H107		C109	
Cycle type	Hot		Hot		Hot		Cold	
Fuel type	D2		D2		D2		D2	
Catalyst	Yes		Yes		Yes		Yes	
Cycle part	P1	P2	P1	P2	P1	P2	P1	P2
Primary Filter PM (mg)	0.262	0.597	0.289	0.578	0.269	0.569	0.922	0.831
Secondary Filter PM (mg)	0.061	0.141	0.058	0.134	0.058	0.134	0.185	0.183
Total Filter PM (mg)	0.323	0.738	0.347	0.712	0.327	0.703	1.107	1.014
Total grams of PM (g)	0.1680	0.3748	0.1806	0.3612	0.1700	0.3634	0.5792	0.5281
PM/ Mile	0.0996	0.0968	0.1067	0.0932	0.1009	0.0938	0.3421	0.1361
Distance (mile)	1.6867	3.8719	1.6926	3.8755	1.6848	3.8742	1.6931	3.8802
Test Total PM Emission	0.0976		0.0973		0.0960		0.1987	

Test Run No.	H110		H111		H112		H113		H114	
Cycle type	Hot		Hot		Hot		Hot		Hot	
Fuel type	20% REE + 80% D2		20% REE + 80% D2		20% REE + 80% D2		50% REE + 50% D2		50% REE + 50% D2	
Catalyst	Yes		Yes		Yes		Yes		Yes	
Cycle part	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
Primary Filter PM (mg)	0.370	0.712	0.368	0.743	0.346	0.702	0.399	0.764	0.354	0.709
Secondary Filter PM (mg)	0.076	0.173	0.092	0.169	0.097	0.155	0.084	0.193	0.09	0.142
Total Filter PM (mg)	0.446	0.885	0.460	0.912	0.443	0.857	0.483	0.957	0.444	0.851
Total grams of PM (g)	0.2332	0.4612	0.2405	0.4727	0.2313	0.4395	0.2518	0.4938	0.2317	0.4330
PM/ Mile	0.1385	0.1191	0.142	0.122	0.1366	0.1136	0.1493	0.1273	0.1373	0.1118
Distance (mile)	1.6838	3.8724	1.6937	3.8746	1.6933	3.8688	1.6865	3.8790	1.6875	3.8730
Test Total PM Emission	0.1250		0.1281		0.1206		0.1340		0.1195	

Test Run No.	H115		H117		H118		H119	
Cycle type	Hot		Hot		Hot		Hot	
Fuel type	50% REE + 50% D2		100% REE		100% REE		100% REE	
Catalyst	Yes		Yes		No		No	
Cycle part	P1	P2	P1	P2	P1	P2	P1	P2
Primary Filter PM (mg)	0.343	0.664	0.352	0.712	1.666	1.606	0.794	1.327
Secondary Filter PM (mg)	0.077	0.137	0.100	0.166	0.314	0.336	0.186	0.263
Total Filter PM (mg)	0.420	0.801	0.452	0.878	1.980	1.942	0.980	1.590
Total grams of PM (g)	0.2189	0.4077	0.2339	0.4476	1.0352	1.0044	0.5117	0.8280
PM/ Mile	0.1296	0.1054	0.1384	0.1156	0.6137	0.2597	0.3026	0.2142
Distance (mile)	1.6890	3.8681	1.6900	3.8720	1.6868	3.8675	1.6910	3.8655
Test Total PM Emission	0.1128		0.1225		0.3672		0.2411	

Test Run No.	H120		H121		H122		H123		H124	
Cycle type	Hot		Hot		Hot		Hot		Hot	
Fuel type	100% REE		100% REE		20% REE + 80% D2		20% REE + 80% D2		20% REE + 80% D2	
Catalyst	No		No		No		No		No	
Cycle part	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
Primary Filter PM (mg)	0.748	1.292	0.717	1.226	0.571	0.981	0.559	0.951	0.55	0.97
Secondary Filter PM (mg)	0.161	0.271	0.139	0.283	0.136	0.213	0.132	0.194	0.143	0.195
Total Filter PM (mg)	0.909	1.563	0.856	1.509	0.707	1.194	0.691	1.145	0.693	1.165
Total grams of PM (g)	0.4747	0.8107	0.4636	0.7692	0.3692	0.6082	0.3604	0.5871	0.3615	0.5975
PM/ Mile	0.2813	0.2098	0.2748	0.1990	0.2184	0.1569	0.214	0.1518	0.2144	0.1546
Distance (mile)	1.6875	3.8642	1.6870	3.8653	1.6905	3.8764	1.6841	3.8676	1.6861	3.8648
Test Total PM Emission	0.2315		0.2220		0.1756		0.1707		0.1728	

Test Run No.	H125		H127		H128		H129	
Cycle type	Hot		Hot		Hot		Hot	
Fuel type	D2		D2		D2		D2	
Catalyst	No		No		No		No	
Cycle part	P1	P2	P1	P2	P1	P2	P1	P2
Primary Filter PM (mg)	0.550	0.889	3.711	1.405	0.562	0.989	0.528	0.974
Secondary Filter PM (mg)	0.126	0.174	0.572	0.289	0.135	0.205	0.171	0.227
Total Filter PM (mg)	0.676	1.063	4.283	1.694	0.697	1.194	0.699	1.201
Total grams of PM (g)	0.3524	0.5515	2.2410	0.8640	0.3642	0.6223	0.3654	0.6142
PM/ Mile	0.2090	0.1426	1.3251	0.2233	0.2164	0.1608	0.2171	0.1590
Distance (mile)	1.6861	3.8675	1.6912	3.8692	1.6830	3.8700	1.6831	3.8629
Test Total PM Emission	0.1628		0.5584		0.1777		0.1766	

Test Run No.	H130		H131		H132		H133		
Cycle type	Hot		Hot		Hot		Hot		
Fuel type	50% REE + 50% D2		50% REE + 50% D2		50% REE + 50% D2		D2		
Catalyst	No		No		No		No		
Cycle part	P1	P2	P1	P2	P1	P2	P1	P2	
Primary Filter PM (mg)	0.788	1.283	0.737	1.282	0.731	1.213	0.544	0.931	
Secondary Filter PM (mg)	0.168	0.289	0.189	0.262	0.159	0.273	0.144	0.199	
Total Filter PM (mg)	0.956	1.572	0.926	1.544	0.890	1.486	0.688	1.130	
Total grams of PM (g)	0.4998	0.8133	0.4863	0.7932	0.4662	0.7683	0.3592	0.5840	
PM/ Mile	0.2969	0.2106	0.2890	0.2051	0.2769	0.1985	0.2132	0.1512	
Distance (mile)	1.6834	3.8618	1.6827	3.8674	1.6836	3.8705	1.6848	3.8624	
Test Total PM Emission	0.2368		0.2305		0.2223		0.1700		

